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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This study is focused on the development of model systems to study interactions between the immune and nervous systems that alter the progress of infectious diseases of the central nervous system (CNS). A temperature-sensitive (ts) mutant of vesicular stomatitis virus (VSV), tsG31-KS5 VSV, intracerebrally inoculated into BALB/c (+/+) or Swiss outbred mice yielded an asymptomatic and persistent infection of the CNS. BALB/c athymic nude (nu/nu) mice infected with tsG31-KS5 VSV, however, all perished within 26 days of infection. The nude mice were afflicted with a slowly progressing CNS disorder, with symptoms including lethargy, curvature of the spine, hind-leg paralysis and other neurological disorders. Reconstitution of nude mice with 5×10^6 syngeneic T lymphocytes, 24 hr prior to their infection with ts VSV, led to at least 70% of the animals surviving, and protection was mediated without a robust humoral antibody response.

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ANNUAL REPORT

The Role of Neuropeptides in Persistent Virus Infections of the Central Nervous System

ONR Contract No. N00014-87-K-0217

A. Introduction

During the past decade significant advances have been made in our understanding of the physiological influences of several neuropeptides, their surprising wide-distribution in non-nervous tissues, and the potential link between these neuropeptides and the immune system. Although to date most studies are preliminary in nature, there already remains little question that subtle changes in neuroendocrine function may play an important role in the host-parasite relationship associated with infectious diseases mediated by viruses.

Central to future advances in our knowledge of the potential interactions between the nervous and immune systems of the host--as a mediator of health and disease--is the development of model systems of infectious diseases that can discriminate between causative and casual relationships. To this end, we have focused the first year of this study on the clinical disease caused by intracerebral inoculation of certain ts mutants of VSV in both BALB/c (+/+) and BALB/c athymic nude (nu/nu) mice. This model system should allow a comparison of immune competent (BALB/c) and immune deficient (BALB/c nude) animals in warding off a progressive CNS disease, the determination of the ability of immune reconstitution (BALB/c nude plus syngeneic T lymphocytes) to alter the clinical course of CNS disease, and the potential modification of the CNS disease by certain neuropeptides that have been implicated in immune function.

B. Research Results

1. Survival of BALB/c (+/+) and (nu/nu) to an intracerebral infection with tsG31-KS5 VSV.

Balb/c nude mice inoculated with tsG31-KS5 VSV all expired by 26 days after infection (Fig. 1). Before death, all the virus infected nude mice suffered a severe CNS disorder with symptoms including lethargy, loss of appetite, wasting, curvature of the spine, and hind-limb paralysis. Nude mice mock-infected with the carrier alone did not experience any symptoms of the CNS disease

and remained healthy for 60 days after the injection at which time the experiment was terminated.

2. Antibody and virus in persistently infected mice.

Nude mice reconstituted with syngeneic T-lymphocyte-enriched splenocytes, however, were relatively refractory to the CNS infection in that over 90% survived for 20 days and 70% lived beyond 25 days (Table 1). Animals that survived remained healthy for at least 60 days after infection, and nude mice reconstituted with either 5×10^6 or 5×10^7 syngeneic T-lymphocytes were provided with a similar degree of protection.

To discern if the immune systems of nude mice had been completely restored with 5×10^6 T lymphocytes, reconstituted nude mice that had been infected for 20 days with tsG31-KS5 VSV were challenged with WT VSV. In Balb/c (+/+) mice, WT VSV infection was fatal to all mice by 4 days of infection. WT VSV, however, was not lethal to any Balb/c (+/+) mice that had been infected with tsG31-KS5 VSV for 20 days before inoculation with WT VSV. Analogous to normal mice, T lymphocyte reconstituted nude mice inoculated with WT VSV all died by 4 days of infection. When T lymphocyte reconstituted nude mice infected for 20 days with tsG31-KS5 VSV were inoculated with WT VSV, only 20% survived the WT VSV challenge. The sera, from the T lymphocyte reconstituted nude mouse that survived the WT VSV challenge, had a VSV neutralizing antibody titer greater than $1:10^4$. Injection of 5×10^6 T lymphocytes into nude mice protected them from the temperature-sensitive VSV induced CNS disease, but their immune functions were not completely restored since the majority did not survive the WT VSV challenge.

Like earlier studies with ts VSV and Swiss outbred mice, VSV could not be detected in the CNS of BALB/c (+/+) mice, even 5-days post-infection (Table 1). However, VSV could easily be isolated from the CNS of almost all nude mice and even those reconstituted with 5×10^6 syngeneic T lymphocytes (Table 1). The fact that nude mice reconstituted with 5×10^6 syngeneic T lymphocytes were able to survive with significant levels of VSV in their CNS, although they did not mobilize a lasting humoral antibody response suggested that cellular immunity may be the most influential factor in the enduring host-parasite relationship.

3. Titration of anti-VSV antibody in nude and normal mice.

After 5 days of infection, immune responses were detected in 8 out of 10 nude mice reconstituted with 5×10^6 T lymphocytes (Table 2). Compared to Balb/c (+/+) mice infected with tsG31-KS5 VSV for 5 days, the neutralizing antibody response of T lymphocyte reconstituted nude mice was weak. Only one mouse reconstituted with 5×10^6 T lymphocytes had a neutralizing antibody

titer equal to Balb/c (+/+) mice responses. A late humoral response was elicited in some of the nude mice reconstituted with 5 x 10^6 T lymphocytes, however, only 30% of the nude mice infected for 20 or 30 days had antibody responses comparable to Balb/c (+/+) mice. This correlates to the fact that only 20% of the T lymphocyte reconstituted nude mice infected for 20 days with tsG31-KS5 VSV survived the WT VSV challenge.

Only 4 of 10 tsG31-KS5 VSV infected nude mice that did not receive T lymphocytes had detectable neutralizing antibody in their sera, and none had a late humoral response against the virus (Table 2). When nude mice were injected with 5 x 10^7 T lymphocytes, 70% of the mice survived a tsG31-KS5 VSV infection, which is similar to the results obtained when nude mice were reconstituted with 5 x 10^6 T lymphocytes (data not shown). Nude mice receiving 5 x 10^7 T lymphocytes had vigorous neutralizing antibody responses against tsG31-KS5 VSV (Table 2). In fact, in the nude mice, reconstituted with 5 x 10^7 T lymphocytes, early and late humoral antibody responses, against tsG31-KS5 VSV, equaled the Balb/c (+/+) mice antibody responses against the virus. The high titers of neutralizing antibody, however, were not required for protection from the CNS disease, since most of the nude mice reconstituted with 5 x 10^6 T lymphocytes and infected for 30 days did not have a vigorous late humoral antibody response, yet survived the tsG31-KS5 VSV infection as well as nude mice reconstituted with 5 x 10^7 T lymphocytes.

When sera from nude mice, both those receiving T lymphocytes and those not reconstituted, were tested for antibody that reacted with tsG31-KS5 VSV in SPRIA, all the sera tested positive (data not shown). A correlation existed between the quantity of neutralizing antibody and the amount of total antibody that bound VSV. The sera of nude mice with elevated titers of neutralizing antibody bound 3 to 5 times more radiolabeled antibody in the SPRIA than did the sera from mice that were negative for neutralizing antibody. Antibody detected by SPRIA could have been non-neutralizing antibody or neutralizing antibody not detected by the plaque reduction assays.

4. Titration of VSV in the CNS of BALB/c and nude mice.

By 5 days of infection with tsG31-KS5 VSV, infectious VSV was difficult to isolate from the brains of persistently infected Balb/c (+/+) mice (Table 1). Infectious VSV, however, was readily isolated from nude mice reconstituted with 5 x 10^6 T lymphocytes one day before infection or from nude mice only inoculated with the virus. In nude mice reconstituted with 5 x 10^6 T lymphocytes and infected for 20 or 30 days, VSV was retrieved only from the CNS of animals that had relatively minor amounts of antibody or no neutralizing antibody (Table 3). Infectious VSV was rarely recovered from nude mice that were reconstituted with 5 x 10^7 T lymphocytes and infected for more than 10 days. The

strong neutralizing antibody responses in Balb/c (+/+) mice and in nude mice reconstituted with 5 x 10^7 T lymphocytes probably made recovery of infectious VSV difficult. All virus isolated from any of the mice was neutralized by antibody made against WT VSV.

Infectious VSV was not isolated from the spleens, livers or sera of any of the mice in Table 3, except for the spleens of 2 nude mice that had not received T lymphocytes and had been infected for 5 days. Less than 1 x 10^3 PFU (plaque forming units) of infectious VSV per spleen were found. The virus isolated from the spleens was neutralized by antibody against WT VSV. The persistent infection, therefore, largely appeared to be limited to the CNS and was not systemic.

5. Characterization of the cloned VSV isolated from the CNS of nude mice.

Since infectious VSV was readily isolated from nude mice that were reconstituted with 5 x 10^6 T lymphocytes, VSV persisting in the CNS for 20 days was characterized. The brains of nude mice, reconstituted with 5 x 10^6 T lymphocytes and one day later infected with tsG31-KS5 VSV, were removed after 20 days of infection and virus clones were isolated and plaque purified. To determine if the clones could asymptomatically persist in normal mice, the CNS clone viruses were inoculated into Swiss outbred mice. The results of eight clones from the brains of 4 different mice (BP1A and BP1B are from the same mouse, BP2A and BP2B are from the same mouse, etc.) are shown in Table 4. All the CNS isolated clones produced more aggressive diseases than tsG31-KS5 VSV in normal mice. In fact, most of the CNS isolated clones when infected into the normal mice were lethal to all the animals. The disease produced by the brain isolated VSV, however, was not like WT VSV infection. WT VSV killed all the mice by 4 days of infection, whereas the brain isolated clones produced a slowly progressing, degenerative disease that generally did not kill the mice for at least 10 days. The brain isolated virus was also less temperature-sensitive than tsG31-KS5 VSV (Table 4). Induction of the more aggressive disease by the brain isolated VSV could ostensibly be attributed to the brain isolated VSV being less temperature-sensitive than tsG31-KS5 VSV.

6. Inoculation of Swiss outbred mice with pooled VSV isolated from the brains of nude mice.

Selection of CNS isolates that caused more aggressive diseases and that were less temperature-sensitive might have occurred when the VSV was cloned, thus the CNS clones may not have been indicative of most of the viruses in the brain pool. Swiss outbred mice, therefore, were inoculated with the virus of four brain pools from which the cloned viruses were derived. Similar to the CNS cloned isolates, the total brain pools also

produced more aggressive diseases than the original tsG31-KS5 VSV (Fig. 2).

7. The modulating influence of β-endorphin.

Similar to our earlier observations with neurotensin and Swiss outbred mice, a single intracerebroventricular injection of 100 ng of β-endorphin in BALB/c (+/+) mice, 24 hr prior to an inoculation with 1 x 10^4 PFU of tsG31-KS5 VSV, dramatically altered the course of clinical disease. The introduction of β-endorphin caused an aggressive CNS disease leading to the death of 70% of the animals within 15 days while only 3% of the mice infected with only the ts VSV died (Fig. 3).

The introduction of β-endorphin 24 hr prior to an intracerebral infection with the ts VSV, however, did not alter the progression of CNS disease in nude mice (Fig. 3). The inability of the neuropeptide to alter the course of CNS disease in nude mice may have been a reflection of a lack of target cells for β-endorphin in the athymic animals. Further experiments with reconstituted nude mice will be needed to assess the basis for this observation.

C. SUMMARY

Nude mice have been used as hosts in many viral model systems, since the role of T cells in the animals defense against the infection can be evaluated. Infection of nude mice with tsG31-KS5 VSV induced a progressively degenerative CNS disease that was lethal to all the animals by 26 days of infection. In contrast, tsG31-KS5 VSV inoculated into normal mice, either Balb/c (+/+) or Swiss outbred, produced an asymptomatic, persistent infection. Although, the immune cell activity may have cleared the VSV from other organs in nude mice, immune cells were not effective in clearing the tsG31-KS5 VSV from the CNS of the nude mice.

A T cell-dependent immune response was required to protect mice from the tsG31-KS5 VSV infection, since nude mice survived the infection and remained disease free when they were reconstituted with syngeneic T lymphocytes.

Studies to determine if tsG31-KS5 VSV induces status spongiosus in normal and/or nude mice will help determine if the status spongiosus, in a manner we previously established with certain ts VSV mutants in outbred mice, is a requisite for the CNS disease. Histopathological evaluation of nude mice reconstituted with T lymphocytes and persistently infected with tsG31-KS5 VSV and of nude mice only infected with the VSV, will determine if T lymphocytes can protect the animals from the spongiform changes

and other neurological lesions that may cause the neurological disease.

How the injected T lymphocytes functioned to protect the nude mice was not clear. The vigorous humoral response elicited in nude mice reconstituted with 5×10^7 T lymphocytes did not seem to be important in protection of the nude mice from the CNS disease induced by the persistent infection, since many nude mice reconstituted with 5×10^6 T lymphocytes did not have a detectable humoral response after 10 days yet the animals remained healthy. Nude mice reconstituted with 5×10^7 T lymphocytes plausibly had both primary and secondary humoral responses against the tsG31-KS5 VSV, whereas most of the nude mice receiving 5×10^6 T lymphocytes probably only had primary immune responses against the VSV. The injected T lymphocytes probably were not directly responsible for protection of the nude mice from the CNS disease.

Since VSV was not consistently found outside the CNS in nude mice not reconstituted with T lymphocytes, an immune mechanism, possibly NK cell activity, must have been functioning in nude mice that cleared the virus from organs other than the CNS. Other viruses have been shown to persist in the CNS after being cleared from the rest of the animal. In normal mice infected with lymphocytic choriomeningitis virus (LCMV), adoptive transfer of specific cytotoxic T cells cleared the virus from the animals except for their CNS which remained persistently infected. A T cell product, such as an interferon or a lymphokine, which could cross the blood-brain barrier more easily than cells may be responsible for the protection of nude mice reconstituted with T lymphocytes from the CNS disease.

The viruses that persisted in the CNS of the T lymphocyte reconstituted nude mice were less temperature-sensitive and caused a more aggressive disease when inoculated into normal mice than tsG31-KS5 VSV. This phenotype may be necessary for the VSV to persist in the CNS of the host. The T lymphocytes injected into nude mice may alter the biochemical nature of the VSV, thus inhibiting the ability of viruses to induce the CNS disease. Since infectious VSV can be readily isolated from the nude mice reconstituted with 5×10^6 T lymphocytes, the biochemical nature of the long term persistent viruses can be evaluated and compared to VSV isolated from nude mice that have not been reconstituted.

D. PUBLICATIONS

Doll, S.C., and Johnson, T.C. 1988. Reconstitution with T lymphocytes protects nude mice from a central nervous system disorder induced by a temperature-sensitive vesicular stomatitis virus. J. Gen Virol. 69: (in press).

Doll, S.C., and Johnson, T.C. 1988. A study of persistent viral infections using nude mice and a temperature-sensitive mutant of vesicular stomatitis virus. N.Y. Acad. Sci. (in press).

Doll, S.C., and Johnson, T.C. 1988. β-endorphin alters a viral induced central nervous system disease in normal but not in nude mice. (submitted for publication).

Doll, S.C., and Johnson, T.C. 1988. The nuclear protein of vesicular stomatitis virus isolated from the brains of nude mice is responsible for abated viral RNA synthesis at the normal body temperature of mice. (submitted for publication).

Table 1. ANTIBODY AND VSV IN PERSISTENTLY INFECTED MICE

	No. of T-Cells	Days Post-Infection	Neutralizing Antibody	VSV in Brain
BALB/C (nu/nu)	0	5	4/10	10/10
		10	0/4	7/7
		20	0/4	4/4
	5×10^6	5	8/10	10/10
		10	2/4	9/10
		20	1/5	4/4
	5×10^7	5	8/10	10/10
		10	4/4	2/10
		20	6/6	0/6
BALB/c (+/+)	-	5	-	0/6
	-	10	-	0/5
	-	20	-	0/10

Mice (nu/nu) were reconstituted with the indicated number of syngeneic T lymphocytes and 24 hr later inoculated with 10^4 tsG31-KS5 VSV. After 5, 10, or 20 days of infection the animals were sacrificed and their sera were measured for neutralizing antibody to VSV by a plaque reduction assay. Animals were scored positive if any neutralizing antibody was detected. VSV in brain was determined by plaque assays of brain tissue homogeneates on BHK cell monolayers incubated at 31°C, a permissive temperature for tsG31-KS5 VSV, and identification of the virus as VSV by antibody neutralization tests.

TABLE 2. TITRATION OF ANTIBODY IN NUDE AND NORMAL MICE

No. of T-Cells Injected*	DAY PI	NEUTRALIZING ANTIBODY** No. Mice Positive / Total Mice	50% Neutralization Titers of Those Positive
Balb/c Mice (nu/nu)			
0	5	4/10	100(3), 10^4
	10	0/10	-
	20	0/10	-
5×10^6	5	8/10	10(3), 100(3), 10^3, 10^5
	10	3/10	10(2), 100
	20	7/10	10(2), 100, 10^3, 10^6(3)
	30	4/10	10, 10^6(3)
5×10^7	5	8/10	10, 100(2), 10^3, 10^4(4)
	10	10/10	100(3), 10^3(3), 10^4(3), 10^5
	20	10/10	100, 10^5, 10^6(8)
	30	10/10	100, 10^6(7), 10^7(2)
Balb/c (+/+) Mice			
	5	4/4	10^4(2), 10^5(2)
	10	4/4	10^3(2), 10^4, 10^5
	20	4/4	10^5, 10^6(3)
	30	4/4	10^6, 10^7(3)

*Nude mice were reconstituted with either 5×10^6 T lymphocytes or 5×10^7 T lymphocytes and 1 day later infected with tsG31-KS5 VSV or only inoculated with virus.

**Neutralizing antibody against VSV was determined by plaque reduction assays.

TABLE 3. ISOLATION OF INFECTIOUS VSV FROM NUDE MICE

No. of T Cells Injected*	DAY PI	VSV BRAIN TITER (PFU) No. Mice Positive Total	Ave. PFU in Positive mice
Balb/c (nu/nu)			
0	5	10/10	1.5×10^4
	10	10/10	5.3×10^4
	20	10/10	2.1×10^6
5×10^6	5	10/10	4.2×10^3
	10	9/10	4.6×10^4
	20	6/10	4.3×10^3
	30	7/10	1.2×10^5
5×10^7	5	10/10	3.8×10^4
	10	2/10	150
	20	0/10	-
	30	0/10	-
Balb/c (+/+)			
	5	0/10	-
	10	0/10	-
	20	0/10	-

*Nude mice were reconstituted with 5×10^6 T lymphocytes or 5×10^7 T lymphocytes, then 1 day later infected with tsG31-KS5 VSV or only inoculated with the virus.

Table 4. CHARACTERIZATION OF CLONED VSV ISOLATED FROM THE CNS OF MICE

VIRUS*	SURVIVAL** (%)	PARALYZED (%)	PFU § ($39^{o}C/31^{o}C$)
WT	0	0	2×10^{0}
tsG31	97	10	9×10^{-4}
BP1A	0	30	2×10^{-2}
BP1B	0	40	2×10^{-2}
BP2A	0	80	2×10^{-2}
BP2B	60	90	7×10^{-2}
BP3A	60	100	2×10^{-2}
BP3B	0	40	1×10^{-2}
BP4A	50	60	3×10^{-2}
BP4B	0	30	1×10^{-1}

*The brain isolated VSV was double cloned by plaque purification

**The clones were inoculated into groups of 10 Swiss outbred mice and the percent survival was determined after 20 days of infection.

§The temperature-sensitivity of the VSV was determined by comparing plaque assays done at $39^{o}C$ and $31^{o}C$.

FIGURE LEGENDS

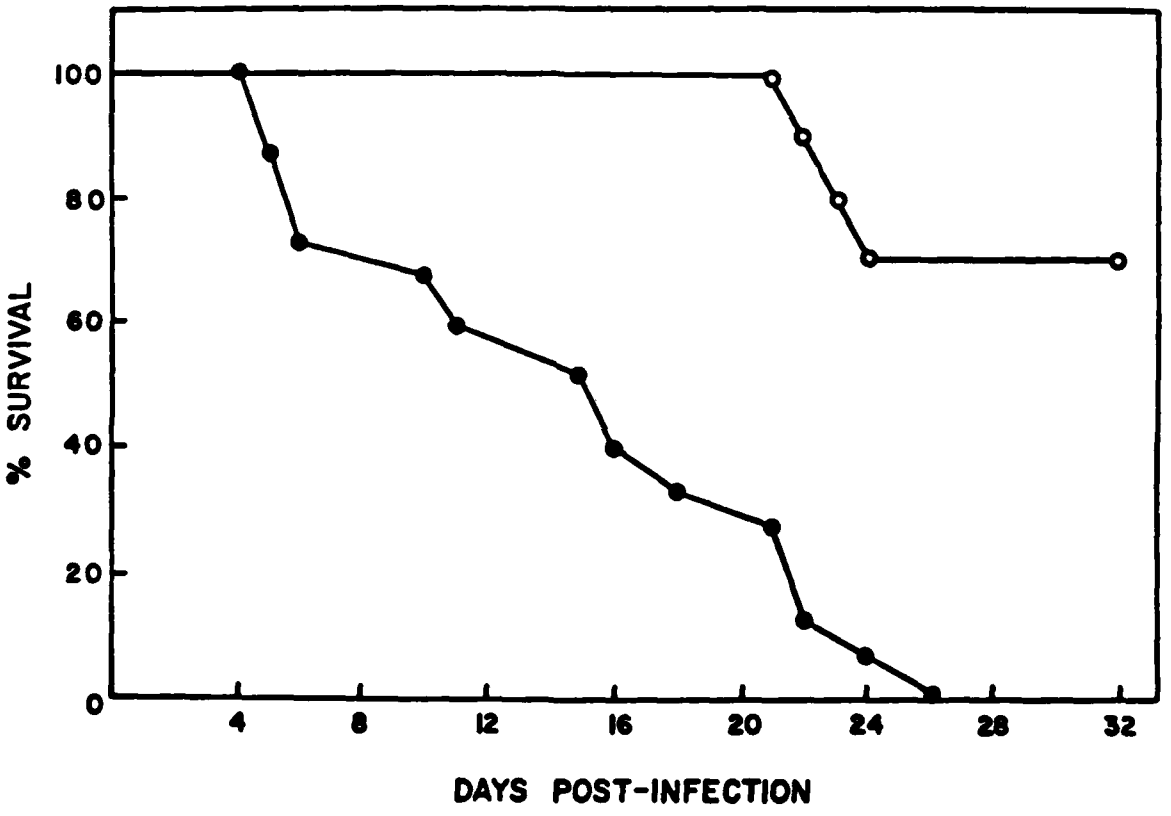
Fig. 1. Protection of Balb/c nude mice from CNS disease, caused by tsG31-KS5 VSV with syngeneic splenic lymphocytes. Ten nude mice were infected with tsG31-KS5 VSV (●) or 15 nude mice were reconstituted with 5 x 10^6 T lymphocytes and 1 day later inoculated with the virus (O).

Fig. 2. Survival of Swiss outbred mice intracerebrally infected with VSV recovered and cloned from the brains of tsG31-KS5 VSV infected nude mice. Nude mice were reconstituted with 5 x 10^6 T lymphocytes and 1 day later inoculated with tsG31-KS5 VSV. After 20 days of infection, the mice were sacrificed and VSV was isolated from their CNS. The brain isolated VSV (BP VSV) from 4 different animals, or tsG31-KS VSV, were inoculated into groups of ten Swiss outbred mice infected with tsG31-KS5 VSV:

(tsG31-KS5 VSV  **, BP1 VSV**  **, BP2 VSV** **, BP3 VSV** **, BP4 VSV**  **).**

Fig. 3. Mice received a single intracerebroventricular injection of 100 ng of β-endorphin in 10 μl of sterile distilled water (**shaded bars**) or with 10 μl of sterile distilled water alone (**open bars**). 24 hr later all mice were infected with 10^4 plaque forming units (PFU) of tsG31-KS5 VSV. (**r**), nude (nu/nu) mice reconstituted with 5 x 10^6 syngeneic T-lymphocytes one day prior to their injection with VSV.

FIGURE 1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DAYS POST-INFECTION

FIGURE 2

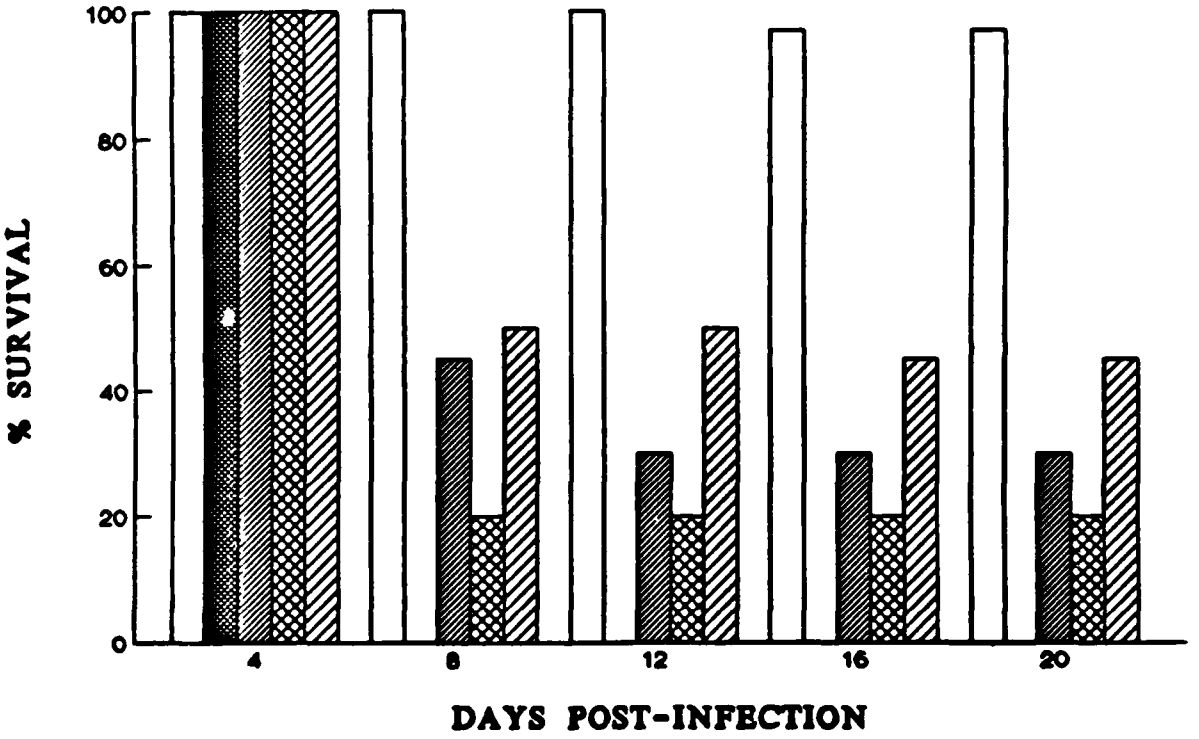
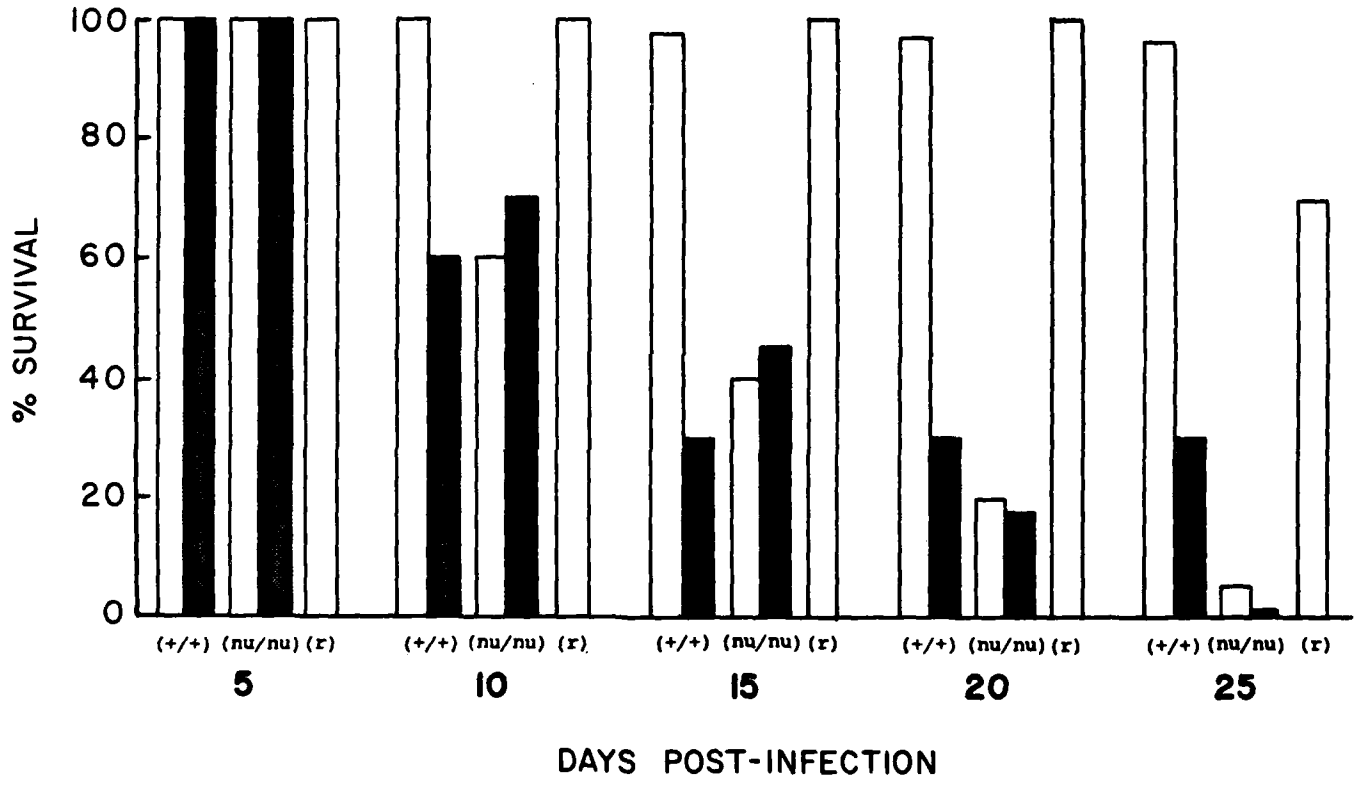


FIGURE 3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DAYS POST-INFECTION

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